

Effect of Potassium Sorbate on Salmonellae, *Staphylococcus aureus*, *Clostridium perfringens*, and *Clostridium botulinum* in Cooked, Uncured Sausage

R. B. TOMPKIN, L. N. CHRISTIANSEN, A. B. SHAPARIS, AND H. BOLIN

Swift & Company, Research and Development Center, Oak Brook, Illinois 60521

Received for publication 26 April 1974

Skinless precooked, uncured sausage links with and without potassium sorbate (0.1% wt/wt) were inoculated with salmonellae, *Staphylococcus aureus*, *Clostridium perfringens*, and *Clostridium botulinum* and held at 27 C to represent temperature abuse of the product. Total counts of uninoculated product showed that the normal spoilage flora was delayed 1 day when sorbate was present. Growth of salmonellae was markedly retarded by sorbate. Growth of *S. aureus* was delayed 1 day in the presence of sorbate, after which growth occurred to the same level as in product without sorbate. *C. perfringens* declined to below detectable levels within the first day in product with and without sorbate. Sorbate retarded the growth of *C. botulinum*. Botulinal toxin was detected in 4 days in product without sorbate but not until after 10 days in product with sorbate.

Sorbic acid and its salts are used in many commercially prepared foods for preventing spoilage. Sorbic acid is selective in its antimicrobial activity. For example, it is a very effective inhibitor of yeasts in cucumber fermentations and yet permits the normal development of lactic acid-producing bacteria, except in those cases of high sorbic acid levels combined with a high initial brine content (3). Sorbic acid has been added to culture media for the selective isolation of catalase-negative lactobacilli and clostridia (5, 7). All strains of *Clostridium botulinum* types A and B tested by York and Vaughn (8) were found to grow within 7 days at 35 C in beef liver infusion containing 3.0% sorbic acid. Hansen and Appelman (6) reported that sorbic acid neither inhibited nor stimulated the growth of *C. botulinum* in culture media.

The observations that sorbic acid is selective in its antimicrobial activity and lack of inhibition for *C. botulinum* form the basis for a very restrictive use of sorbic acid and its salts in meat products. The only approved use consists of dipping the casings for stuffed dry sausage. This application inhibits mold growth on the surface of the sausages during the long period they are held in drying rooms. Sorbate cannot be added to the meat portion of pizza pies even though it is permitted in the cheese and crust.

Spoilage by molds results in significant loss of meat products at the retail and consumer levels. Addition of sorbates to the meat would reduce this loss of food. This study was designed to determine the effect of potassium sorbate on *C. botulinum* in cooked sausage in the event the product is temperature abused. Also, the effect of potassium sorbate on the growth of salmonellae, *Staphylococcus aureus*, and *C. perfringens* was examined. The methods of inoculating the product represent recontamination on the surface of the sausages after cooking (salmonellae and *S. aureus*) and spores surviving the cooking process (*C. botulinum* and *C. perfringens*).

MATERIALS AND METHODS

Meat. Cooked, skinless sausage links were used as the test system. The raw meat formula consisted of a mixture of beef and pork (87.95%), water (8.8%), sodium chloride (1.75%), and sugar and spices (1.5%). The fat content of the uncooked links was 43%. One lot of sausages was prepared in which 0.1% potassium sorbate (wt/wt; Mallinckrodt Chemical Works, Lodi, N.J.) was added to the ground meat formula prior to cooking. The links were heated to an internal temperature of 71 C and then frozen until needed. The cooked sausages had a pH of 6.2. The average weight of the links were 20 g each.

Cultures. Five salmonellae cultures (*Salmonella anatum*, *S. infantis*, *S. senftenberg*, *S. choleraesuis*, and *S. newport*) were grown at 37 C in brain heart infusion broth (BHI; Difco). After incubation for 18 h,

the five strains were pooled for inoculation of the sausage. Five strains of *S. aureus* (S-6, 196E, 361, FDA 315, and ATCC 6538) were grown in BHI broth at 37 C and pooled after 18 h. Four of these strains are known to produce one or more of the known enterotoxins. Spores of three strains of *C. perfringens* (NCTC 8239, FDI, and ATCC 3624) were prepared as described by Duncan and Strong (4). The spore crops were harvested by centrifugation, washed several times in sterile distilled water, then resuspended in phosphate buffer (pH 7.0) and pooled to provide equal levels of each of the three strains for inoculation. Spores of five strains of type A (77A, 62A, 33A, 12885A, and 36A) and five strains of type B (9B, 40B, 41B, 51B, and 53B) *C. botulinum* were prepared as previously described (2). A pooled mixture of the 10 spore crops was used for inoculation of the sausage.

Inoculation of meat. Our goal was to achieve an inoculum level of 1,000 *C. botulinum* and 1,000 *C. perfringens* spores per link and 1,000 salmonellae and 1,000 *S. aureus* per g of product. Sausages were inoculated on the outer surface with salmonellae by immersing the sausages in a dilute aqueous suspension of the pooled salmonellae. The sausages were removed from the suspension and allowed to drain. Each sausage link retained approximately 0.1 ml of the suspension and yielded an initial inoculum level of 32 to 38 per g or 640 to 760 per sausage link. The same method of inoculation for *S. aureus* yielded an initial level of less than 30 per g or less than 600 per sausage link. This inoculum level was below the limit of our method for enumerating *S. aureus*.

The mixed spore suspension of *C. botulinum* was heat shocked at 80 C for 15 min and 0.1 ml was injected into the center of the sausage links. This yielded an inoculum level of more than 7,200 per g or 140,000 per sausage link, which was more than anticipated. The mixed spore suspension of *C. perfringens* was heat shocked at 70 C for 20 min before injection. The inoculum level was 320 per g or 6,400 per sausage link.

The above methods were followed for product with and without potassium sorbate. Separate inoculum suspensions of salmonellae and *S. aureus* were used to dip sausages with and without sorbate.

Storage and sampling of product. Samples representing each variable were packed separately in boxes (10 links per box) giving a net weight of about 200 g. Uninoculated control product was included for comparison. All product was stored at 27 C. Three boxes of each variable were removed for analysis at predetermined time intervals. A 90-g sample was removed from each box and blended in a Waring blender with 90 ml of sterile phosphate buffer (0.003 M, pH 7.0) for 1 min. After making appropriate dilutions, viable counts were determined. Total aerobic counts of uninoculated product were made by using standard plate count agar (Difco) with incubation at 27 C for 2 days. Salmonellae were enumerated by spreading onto brilliant green sulfa agar (Difco). *Salmonella*-like colonies were counted after 24 h at 37 C. Representative colonies were transferred into lysine iron agar slants (Difco) and examined for typical reac-

tions. Samples inoculated with *S. aureus* were plated onto Baird-Parker agar (Difco) and examined after 24 h at 37 C. Samples inoculated with *C. perfringens* were plated into SFP agar (Difco), overlaid, and incubated in anaerobic jars at 37 C for 24 h. Counts were made and typical colonies were confirmed by transferring into nitrate-motility medium (1). Samples inoculated with *C. botulinum* were analyzed by a three-tube, most-probable-number technique using peptone colloid (Difco) modified by the addition of 0.1% dextrose, 0.05% ferrous sulfate, and 0.03% sodium thiosulfate. All tubes showing blackening after day 7 at 37 C were assumed to contain *C. botulinum*. Black tubes from the highest dilutions were selected at random and were confirmed by mouse test to contain botulinal toxin. Sausages inoculated with *C. botulinum* were also tested for toxin by centrifuging a portion of the 1:1 suspension of the blended sample. Two white mice were injected with 0.5 ml of the supernatant fluid. A third mouse was injected with 0.5 ml of supernatant which had been boiled for 15 min. Death of the mice receiving the unheated extract and survival of the mouse injected with the boiled extract coupled with the *C. botulinum* viable counts were considered evidence for the presence of botulinal toxin.

RESULTS AND DISCUSSION

Potassium sorbate delayed the growth of the normal bacterial spoilage flora in the uninoculated product during the first day, after which growth was rapid (Table 1). Growth of the salmonellae was markedly retarded by potassium sorbate. This agrees with a report (5) that sorbic acid inhibited salmonellae in culture media. Growth of *S. aureus* was inhibited during the first day, after which growth was rapid. *C. perfringens* declined to below detectable levels in all samples within the first day, independent of the presence or absence of potassium sorbate.

Growth of *C. botulinum* was slower in the product containing potassium sorbate. Also, there was a delay in the development of botulinal toxin. Botulinal toxin was detected in 4 days in product without sorbate, but not until after 10 days in the product with sorbate. The product inoculated with *C. botulinum* had pH values of 6.4 and 7.1 after 4 days in samples with and without sorbate, respectively. A pH of 6.4 is sufficiently high to exclude the possibility that pH was a factor in the inhibition of botulinal growth and toxin production.

These data demonstrate that the addition of 0.1% potassium sorbate to retard mold spoilage does not increase the public health hazard of cooked pork sausage in the event that it becomes temperature abused. To the contrary, the

TABLE 1. Effect of potassium sorbate on microbial growth in cooked pork sausage at 27 C^a

Time (days)	Uninoculated		salmonellae		<i>S. aureus</i>		<i>C. perfringens</i>		<i>C. botulinum</i>		No. toxic/no. tested	
	No sorbate	With sorbate	No sorbate	With sorbate	No sorbate	With sorbate	No sorbate	With sorbate	No sorbate	With sorbate	No sorbate	With sorbate
0	3.2 × 10 ⁴	3.1 × 10 ⁴	3.8 × 10 ¹	3.2 × 10 ¹	<30	<30	3.2 × 10 ²	3.2 × 10 ²	7.2 × 10 ³	7.2 × 10 ³	0/3	0/3
1	1.2 × 10 ⁷	3.2 × 10 ⁴	3.5 × 10 ⁶	8.1 × 10 ²	3.0 × 10 ⁶	1.8 × 10 ⁴	<30	<30	1.5 × 10 ⁴	1.8 × 10 ⁴	0/3	0/3
2	6.9 × 10 ⁸	1.7 × 10 ⁷	6.3 × 10 ⁷	<3.0 × 10 ³	1.2 × 10 ⁸	4.4 × 10 ⁸	<30	<30	2.4 × 10 ⁴	5.3 × 10 ³	0/3	0/3
4	— ^b	—	—	—	—	—	<300	<300	5.1 × 10 ⁶	1.8 × 10 ⁴	3/3	0/3
5	7.0 × 10 ⁸	1.1 × 10 ⁹	1.1 × 10 ⁷	3.0 × 10 ⁴	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	<3,000	<3,000	3.5 × 10 ⁷	3.5 × 10 ⁶	3/3	0/3
10	—	—	—	—	—	—	—	—	5.6 × 10 ⁶	9.1 × 10 ⁷	3/3	0/3
17	—	—	—	—	—	—	—	—	1.3 × 10 ⁶	7.5 × 10 ⁶	3/3	3/3

^a Expressed as viable counts per gram. All viable counts are geometric means of three samples at each sampling time.

^b —, Not tested.

data demonstrate that the addition of potassium sorbate reduces the public health hazard. The observation that botulinal toxin formation was retarded was not expected and should receive additional study. It would be of value to learn if sorbate has a similar effect in other food systems.

ACKNOWLEDGMENTS

This research was supported in part by American Hoechst Corp., Sommerville, N.J., Mallinckrodt Chemical Works, Lodi, N.J., Monsanto Chemical Co., St. Louis, Mo., Pfizer Chemical Co., Brooklyn, N.Y., and Tanower Co., San Diego, Calif.

LITERATURE CITED

1. Angelotti, R., H. E. Hall, M. J. Foter, and K. H. Lewis. 1962. Quantitation of *Clostridium perfringens* in foods. *Appl. Microbiol.* **10**:193-199.
2. Christiansen, L. N., R. W. Johnston, D. A. Kautter, J. W. Howard, and W. J. Aunan. 1973. Effect of nitrite and nitrate on toxin production by *Clostridium botulinum* and nitrosamine formation in perishable canned comminuted cured meat. *Appl. Microbiol.* **25**:357-362.
3. Costilow, R. N., F. M. Coughlin, E. K. Robbins, and W.-T. Hsu. 1957. Sorbic acid as a selective agent in cucumber fermentations. II. Effect of sorbic acid on the yeast and lactic acid fermentations in brined cucumbers. *Appl. Microbiol.* **5**:373-379.
4. Duncan, C. L., and D. H. Strong. 1968. Improved medium for sporulation of *Clostridium perfringens*. *Appl. Microbiol.* **16**:82-89.
5. Emard, L. O., and R. H. Vaughn. 1952. Selectivity of sorbic acid media for the catalase negative lactic acid bacteria and clostridia. *J. Bacteriol.* **63**:487-494.
6. Hansen, J. D., and M. D. Appelman. 1955. The effect of sorbic, propionic, and caproic acids on the growth of certain clostridia. *Food Res.* **20**:92-96.
7. York, G. K., and R. H. Vaughn. 1954. Use of sorbic acid enrichment media for species of *Clostridium*. *J. Bacteriol.* **68**:739-744.
8. York, G. K., and R. H. Vaughn. 1955. Resistance of *Clostridium parbotulinum* to sorbic acid. *Food Res.* **20**:60-65.